

Dexamethasone Concentrations in Ovine Plasma during its Intravenous Infusion, its Relation to the Production of Some Endogenous Hormones, and Some of the Effects on Wool Growth

B. A. Panaretto and A. L. C. Wallace

Division of Animal Production, CSIRO, Ian Clunies Ross Animal Research Laboratory, P.O. Box 239, Blacktown, N.S.W. 2148.

Abstract

Plasma concentrations of dexamethasone have been measured in sheep during an 8-day infusion of dexamethasone-21-phosphate. The dexamethasone concentration profiles generally revealed a reproducible pattern with three phases—a peak during the first 48 h infusion which was followed by falling concentrations during the next 5 days, and a small increase in dexamethasone concentration during the final 24 h infusion was not uncommon. The pattern of dexamethasone concentrations was retained when dosage was arranged in such a way as to infuse increasing quantities of hormonal analogue as infusion progressed. Aspects of the metabolism of the analogue are discussed.

Endogenous thyroxine and cortisol were significantly depressed during infusion. In these experiments wool was completely shed in three out of four animals dosed at a rate of 8.5 mg dexamethasone/kg^{0.75}. The recovery of wool growth to its pretreatment values occurred by about one month after infusion. The consumption of food and body weight increases were satisfactory during the post-infusion period.

Introduction

Depression of wool growth in sheep by the administration of adrenal glucocorticoid hormones was first reported by Lindner and Ferguson (1956) and has been confirmed both for cortisol (Chapman and Bassett 1970; Thwaites 1972) and for some synthetic analogues (Ferguson *et al.* 1965; Panaretto *et al.* 1975). The plasma concentrations of glucocorticoids and the time periods over which these must be maintained in order to inhibit wool growth is not known. Most experimenters have administered hormones over prolonged periods; for example Lindner and Ferguson (1956) injected sheep with cortisone acetate for 10 weeks in order to depress wool growth, but technology at that time did not enable them to determine circulatory concentrations of the hormone. Chapman and Bassett (1970) reported that the retrogression of all components of the epidermis and dermis was maximal when plasma cortisol exceeded 3 µg/100 ml. The protocol of their experiments, however, makes it difficult to determine the time over which this hormonal concentration was present. The fact that high concentrations of cortisol are required to inhibit hair follicles is suggested also by the observations of Singh and Hardy (1975) who reported that 75 µg cortisol/ml culture medium was required over 6 days in order to cause the regression of hair follicles in cultured foetal mouse skin.

The depressant effects on wool growth of a potent analogue of cortisol, dexamethasone [9-fluoro-11,17,21-trihydroxy-16 α-methyl-pregna-1,4-diene-3,20-dione (Arth *et al.* 1958; Sarett 1959)], was reported by Ferguson *et al.* (1965) and of some dexamethasone derivatives by Panaretto *et al.* (1975) but in neither investigation were

plasma concentrations of the hormone or analogues reported. Panaretto *et al.* (1975) also reported much variability in wool growth depression between sheep given identical single intravenous doses of dexamethasone derivatives. These differences varied from animals that shed their complete fleeces, to those that showed no discernible effect on wool growth. At that time it was suggested that differences in absorption, metabolism and excretion could be responsible for the variability in effects observed.

Circulatory concentrations of dexamethasone are reported here during the intravenous infusion of a dexamethasone derivative. Some aspects of the metabolism of the analogue are reported together with relationships between the infused hormone and endogenous cortisol and thyroxine. Finally observations on wool growth in infused sheep are included.

Materials and Methods

Animals

Merino wethers, aged 1½–5 years old, bearing 5–6 cm wool and individually housed in cages in an animal house, were used. Each animal bore a delineated area of 100 cm² on its midside. The sheep were fed 600 g daily of a ration of equal parts of lucerne chaff and oats for the 10–14 weeks before intravenous infusions were made and 1000 g of the same food immediately following the end of infusion. Food was given once daily at approximately 1615 h.

Body weights of the sheep at the start of the infusions are given in Table 1.

Table 1. Body weights and dosages of dexamethasone in sheep in experiments 1 and 2

Experiment	Sheep No.	Body weight		Dexamethasone ^A			
		(kg)	(kg MBW)	Dose (mg)	(mg/kg)	Dose rate (mg/kg MBW)	(mg/h)
1	9374	27.6	13	46.4	1.5	3.6	0.24
	9379	33.6					
	9632	25.5	11.1	93.6	3.8	8.4	0.49
	9684	24.2					
	5132	50	18.1	167	3.5	9.2	0.87
	5034	45					
2	9661	29.6 ±1.5	12.7	62.8	2.1	4.9	See Fig. 2a
	9504						
	9443						
	9597						

^AExpressed in terms of the free alcohol.

Hormone Dosage, Infusion and Blood Sampling

Dexamethasone phosphate (9-fluoro-11,17-dihydroxy-16 α -methyl-pregna-1,4-diene-3,20-dione-21-disodium phosphate) was supplied (by Merck, Sharp and Dohme Pty Ltd, South Granville, N.S.W.) as a white crystalline powder that was freely soluble in water. The dosage of the hormonal analogue is always expressed in terms of the free alcohol and expressed either in terms of body weight or body weight raised to the power 0.75 (called metabolic body weight, MBW). In experiment 1, one-eighth part of the total dose was dissolved in 45 ml of saline daily and evenly infused over 24 h using a Harvard infusion pump (Harvard Apparatus Co. Inc., Mass., U.S.A.). The doses of hormone analogue in experiment 2 were arranged so that 9.4, 15.2 and 38.2 mg dexamethasone were delivered in 90, 90 and 180 ml of saline solution respectively during the first 48, 48–96 and 96–192 h using the same pumping system. Hormone doses were freshly prepared every 24 h and dexamethasone infusion rates of about 0.2, 0.32 and 0.4 mg/h were given.

Infusions and blood sampling were made through polyethylene tubing (i.d. 0.86 mm, o.d. 1.54 mm) (Dural Plastics Pty Ltd, Dural, N.S.W.) inserted into each animal's jugular veins in opposite directions; blood samples were taken at approximately 0900 and 1500 h daily from the cranially directed catheters while infusions were made in the cardiac-directed catheters.

Analytical

The method used was a modification of that described by Hichens and Hogans (1974). Dilutions of standard dexamethasone were made in pooled sheep plasma and 50 μ l of standard or unknown sera was mixed with 350 μ l of 1/1000 antiserum and incubated for 4 h at room temperature. 100 μ l of [3 H]dexamethasone (Amersham, England) was added and the samples left overnight at 4°C.

Free and protein-bound radioactivity were separated by the addition of 50 μ l of 5% charcoal. After 10 min the tubes were centrifuged and 500 μ l of the supernatant was added to 10 ml of scintillator solution and counted.

The antiserum cross reacts with dexamethasone phosphate to the extent that 10% of any circulating dexamethasone phosphate would be measured as free dexamethasone. However, most of the steroid present in plasma appeared to be in the form of free dexamethasone. Hydrolysis of plasma samples with phosphatase at 37°C for 1 h did not increase the concentrations as measured by the assay. The treatment was sufficient to hydrolyse standard solutions of dexamethasone phosphate. Hydrolysis of dexamethasone phosphate during the assay was checked by assaying the same samples with and without the addition of 0.02 M sodium arsenate. No evidence for hydrolysis was found. Thus in the sheep, as in humans (Hare *et al.* 1975), the hydrolysis of dexamethasone phosphate *in vivo* was rapid. The intra-assay coefficient of variation was 4.5% at 35 ng/ml (20 assays); the inter-assay coefficient of variation ranged from 3 to 7% (4 assays).

Thyroxine was measured by the radioimmunoassay for triiodothyronine method of Eastman *et al.* (1975). The hormone was assayed in 10- μ l aliquots of unknown sera and 25 mg sodium salicylate was used to displace thyroxine from thyroxine-binding globulin.

Cortisol was measured by the method of Bassett and Hinks (1969).

Wool Growth

Wool growth was measured in each animal by shearing the wool from the defined areas on their midsides. Each sample was conditioned and weighed and the clean dry weights were measured using the method of Hemsley *et al.* (1973). Control measurements were made for 12 weeks before treatment.

The effects of the hormonal analogue on wool growth were assayed as follows:

Shedding. Shedding of the fleece was that state where the wool fibres were discontinuous and shed from the fibre canal although in parts the separated wool lay on the body surface. Shedding was either complete or regional.

Broken. The fleece as a whole was not shed but contained a large enough proportion of shed fibres among the continuous ones retaining the fleece to constitute an easily discernible 'wool break'.

Results

Plasma Dexamethasone Concentrations

Experiment 1

Plasma dexamethasone concentrations during the 8 days of infusion are illustrated in Fig. 1a. Several features were evident in the results. The plasma concentrations bore little relationship to the rate of infusion of the hormone. For example, the rates of infusion of dexamethasone into sheep 9632, 9684, 5132 and 5034 were comparable on a body weight basis—approximately 3.5–3.8 mg dexamethasone/kg body weight or 8.4–9.2 mg/kg MBW (see Table 1)—but peak concentrations of dexamethasone in these four sheep after approximately 24–48 h infusion varied several-fold (by a factor of 2.5–3.3; 40 v. 130 ng/ml). The rate of infusion of dexamethasone into sheep 9374 and 9379 was approximately 0.4 times its rate of infusion into the other sheep. Plasma dexamethasone concentrations at 24 h infusion were very similar in

sheep 9374, 9379 and 9684 even though the rate of dexamethasone infusion into this last animal was 2.5 times that in the other two.

Another common feature, with the possible exception of sheep 9632, was the tendency for peak dexamethasone concentrations, evident after 24–48 h infusion, to be followed by steadily decreasing concentrations throughout the infusion period to days 5–6 of infusion. After day 5 concentrations were relatively constant around values of 10–20 ng/ml or increased towards the end of the infusion period—for example in sheep 5132 and 5034 (Fig. 1a). Sheep 9632 tended to maintain a relatively constant plasma dexamethasone concentration of 50–60 ng/ml throughout the first 4 days of infusion. Technical infusion difficulties made it impossible to determine events on days 5 and 6 of infusion.

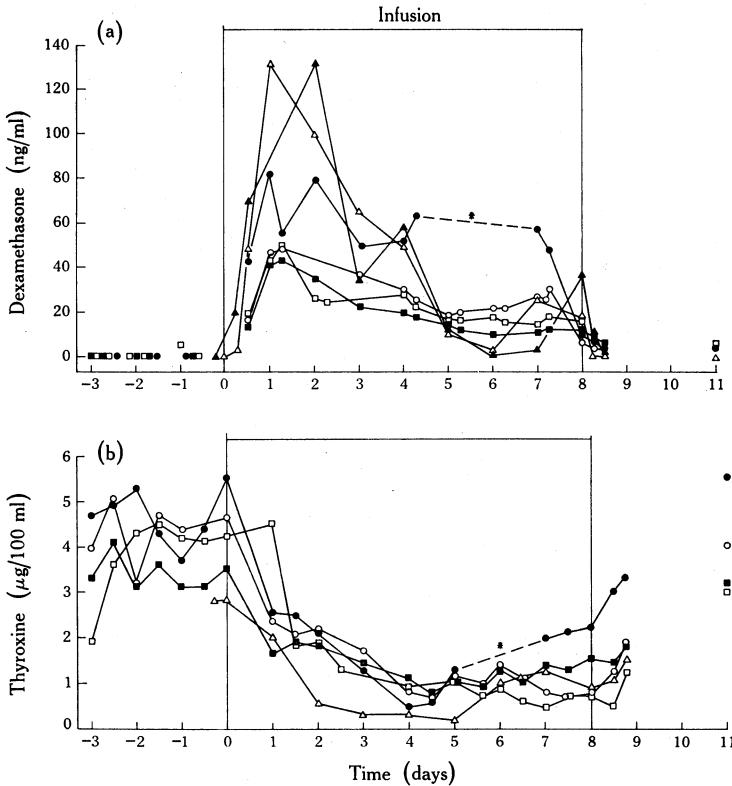


Fig. 1. Plasma (a) dexamethasone and (b) thyroxine concentrations before, during and after the intravenous infusion of dexamethasone for 8 days in experiment 1. Infusions were made at the rate of 4 $\mu\text{g}/\text{min}$ (sheep ■ 9374, □ 9379), 8 $\mu\text{g}/\text{min}$ (sheep ○ 9684, ● 9632) and 14.5 $\mu\text{g}/\text{min}$ (sheep △ 5132, ▲ 5034). Sheep 5034 is not shown in (b). *Difficulties were experienced with infusions at these times and no blood was taken for analysis.

Experiment 2

Dexamethasone concentrations in plasma are shown in Fig. 2a.

Much the same pattern was obtained here with respect to dexamethasone concentrations during infusion as in experiment 1. Peak concentrations were again measured during the first 48 h infusion at a time when the infusion rate of hormonal

analogue was one-half the rate during the last 4 days of the experiment. Peak concentrations again varied 2.5-fold between sheep (130 v. 65 ng/ml). In some cases dexamethasone concentrations began to fall during the first 48 h of infusion and in every case the concentrations during 48–96 h were substantially less than those measured at the peak. Relatively stable concentrations (between 10 and 50 ng/ml) were seen during the last 4 days of infusion. There was again a tendency for some sheep to show increased plasma dexamethasone concentrations during the final 24 h infusion.

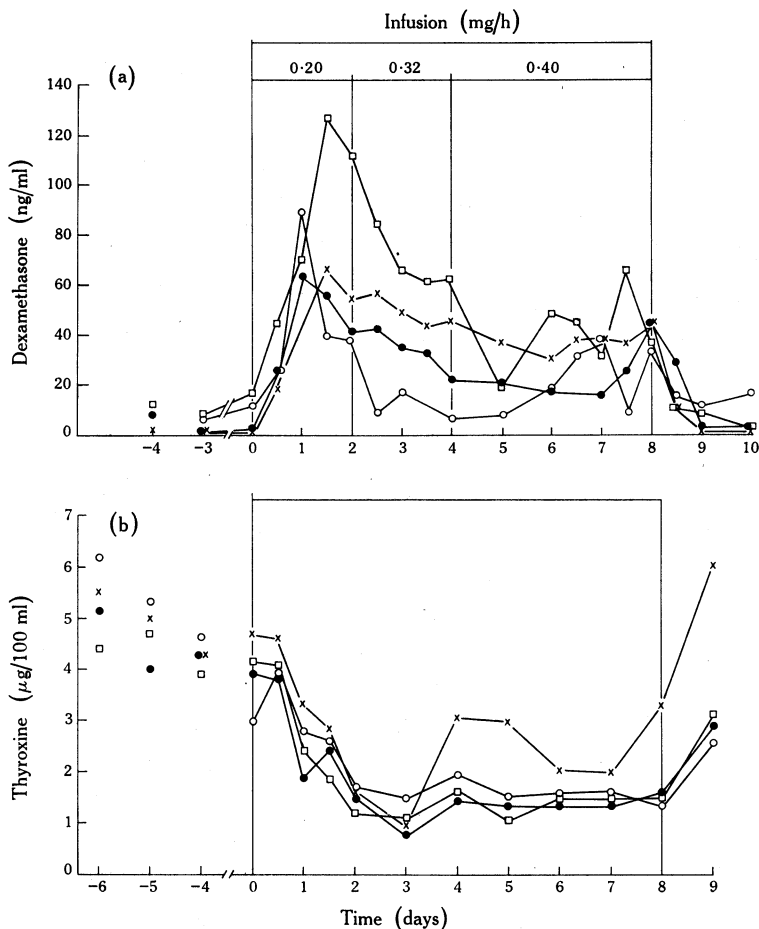


Fig. 2. Plasma (a) dexamethasone and (b) thyroxine concentrations before, during and after the intravenous infusion of dexamethasone for 8 days in experiment 2 (sheep ● 9597, × 9661, ○ 9443 and □ 9504). During the first 48 h of infusion dexamethasone was given at a rate of 0.20 mg/h; between 48 and 96 h the infusion rate was increased to 0.32 mg/h, and over the last 4 days the rate was 0.40 mg/h.

Plasma Thyroxine Concentrations

Experiment 1

Plasma thyroxine concentrations are illustrated in Fig. 1b. A rapid decrease in thyroxine concentrations from a mean pre-infusion value of approximately 40 to

<10 ng/ml on day 4 of infusion was evident. The concentrations in two animals remained below a level of 10 ng/ml during the rest of the infusion period. Values obtained 4 days after infusion were equal to those obtained pre-infusion.

Experiment 2

Pre-infusion values for thyroxine of 40–60 ng/ml were recorded (Fig. 2b). These fell quickly during infusion to values between 10 and 20 ng/ml, approximately 15 ng/ml. All values showed an increase 24 h after the infusion although pre-infusion concentrations had not been reached at that time.

Endogenous Cortisol Concentrations

Experiment 1

The mean plasma cortisol concentrations prior to infusion were 10.4 ± 1.0 µg/l. No endogenous plasma cortisol could be measured during infusion.

Table 2. Wool growth after intravenous infusion of dexamethasone

The sheep were infused for 8 days with dexamethasone at the rate of 1.5 mg/kg body weight (sheep 9374, 9379), or 3.5–3.8 mg/kg body weight (sheep 9632, 9684 and 5132 and 5034). Wool was taken from tattooed areas of 100 cm² on the midsides at 14- or 15-day intervals and values are shown for clean dry wool relative to pre-treatment levels (100). The animals had been fed 600 g of a mixture of lucerne chaff and oats (1:1) before infusion and 1000 g after infusion

Sheep No.	Relative growth of wool at intervals from the end of infusion (days):				
	5	29	34	48	62
9374	72	105	146	189	219
9379	58	45	143	152	160
9632	53	43	125	196	213
9684	72	71	178	200	200
5132	70	23	35	58.5	87
5034	70	10	40	124	202

Wool Growth

Experiment 1

The slow intravenous dose of approximately 8.5 mg dexamethasone/kg MBW inhibited wool growth in all wool fibres in three out of four sheep (see Table 1, sheep 9632, 5034 and 5132) so that, with the subsequent regrowth of new fibres, the whole fleece was shed. The result of this, at 28 days after infusion, was that the old fleece was hanging off the animal in some places and in others laying on the newly emerging wool fibres. The old fleece could easily be removed by hand. In the other sheep there were regional differences in inhibition of fibre growth over the surface of the body. In sheep 9684 wool was shed from the thorax and trunk but enough wool fibres remained unshed over the cervical and lateral thigh regions to preclude the possibility of removing the wool manually. Wool was readily removed from all body regions of sheep 9379, with the exception of the cervical region where the fleece was broken. In sheep 9374, however, no wool could be removed from the animal, since the fleece was retained by unbroken fibres although enough fibres were discontinuous to form an easily discernible 'break'. In all cases new fibre growth was occurring under the shed fleece. This observation was supported by clean dry wool weights obtained at fortnightly intervals from defined areas on the midsides of

the first four sheep included in Table 2. In these four animals, at approximately 34 days after clipping, wool was growing at a rate greater than that during the control period. Wool did not exceed its control growth rate in sheep 5132 and 5034 until approximately 60 and 40 days post-infusion respectively.

Experiment 2

There was no shedding of the whole fleece from any sheep in this experiment. The proportion of shed fibres varied, from a low subjective estimate of fewer than one-half broken in sheep 9443, to 90% broken fibres in sheep 9597.

Food Intake

There were no feed residues in either experiment from both levels of feeding, i.e. 600 or 1000 g food offered.

Body Growth

Experiments 1 and 2

Body weights increased rapidly in all animals when they were given 1000 g food daily. The increase in body weight approximated 1 kg/week and continued at this rate for 10 weeks.

Discussion

The concentrations of dexamethasone in the blood during infusion of the analogue dexamethasone-21-phosphate followed a pattern in which the highest values were recorded during the first 48 h infusion. The high values were followed by a period of prolonged decrease in concentrations as the infusion continued. A similar pattern was also observed when the rate of infusion of analogue during the first 48 h was half that during the last 96 h. The results thus imply at least two or possibly more clearance rates for dexamethasone from the circulation of any individual sheep—a slow clearance rate early during infusion followed by a progressively increasing clearance rate as infusion proceeded. The increases in plasma dexamethasone seen during the final day of infusion in several sheep may have been due to a return to the circulation of dexamethasone hitherto sequestered in a compartment that we could not sample. In a number of species of animals several drugs stimulate their own metabolism during their chronic administration by inducing the formation of enzymes that metabolize them (Conney 1967). This possibility needs to be considered. Induction of drug-metabolizing enzymes is first preceded by a period of enzyme inhibition (Parke 1976). Thus a mechanism that may be operating to elevate plasma dexamethasone concentrations early during infusion may be the inhibition of the metabolizing enzymes already present. Whether or not these processes are occurring in our experiments needs further investigation. Furthermore, the large range of differences in plasma dexamethasone concentrations between sheep given doses of equal size imply different clearance rates between sheep. This is not surprising since Haque *et al.* (1972), using radioactive dexamethasone in humans, reported a range of clearance rates from 222 to 456 l/day. The metabolism of dexamethasone by sheep is currently being studied further at this laboratory and initial results have confirmed the plasma concentration profiles reported here (Leish and Panaretto, unpublished data).

Under the conditions of these experiments 8.5 mg dexamethasone/kg MBW caused complete fleece shedding in three out of four sheep. This appeared to have

been achieved without deleterious effects on the animals' ability to consume feed when the food ration was increased after infusion and to show excellent body weight increases subsequently. Four out of six of the animals exceeded control (i.e. pre-infusion) wool growth approximately 1 month after infusion. Sheep 5034 and 5132 showed a greater depression of wool growth than the other animals on day 29 after infusion and recovery of their wool growth rate was slower.

It is difficult to reconcile plasma dexamethasone concentrations with effects on wool growth. By manipulating the rates of infusing the hormone analogue in experiment 2 similar plasma dexamethasone concentrations were achieved, using an overall dose rate of 2.1 mg/kg, to those in the sheep dosed at a rate of 3.5 mg/kg or higher in experiment 1. With the possible exception of sheep 9632, higher concentrations of dexamethasone were measured during the last 4 days of infusion in experiment 2 than in experiment 1. Yet in no case were all wool fibres inhibited in their growth in experiment 2 and broken fleeces resulted. Not only were different concentrations reached by infusing similar quantities of the analogues into different sheep, but different effects on wool growth were seen at similar concentrations of dexamethasone (see Table 2, sheep 9374 and 9379). Both sheep were growing wool at comparable rates during the control period: 0.751 ± 0.040 and 0.888 ± 0.025 g per 100 cm² per 14 days. Depression in wool growth in sheep 9374 was, however, not as marked as in sheep 9379, and sheep 9374 was the first of the treated sheep to regain its pre-infusion rate of wool growth at approximately 20 days post-infusion.

The importance of a small proportion of continuous fibres in retaining shed fibres in Shetland sheep has been emphasized by Ryder (1971). The importance of the broken fleeces observed in experiment 2 was this: if a small enough proportion of continuous to shed fibres could be achieved, and controlled within narrow limits, then wool harvesting might be done by breaking the few continuous fibres after regrowth was established. Such a strategy would overcome the loss of shed fibres in the paddocks and protection of the defleeced animal from climatic stresses (see, for example, Roberts and McMahon 1972).

The decrease in plasma cortisol was expected and was a result of the depression of adrenal steroid release by the high circulating levels of exogenous steroid. It was unlikely that cortisol interfered with the dexamethasone assay used here.

The effect on thyroid function has been previously described in humans (Wilber and Utiger 1969; Faglia *et al.* 1973; Degroot and Hoyer 1976) and in rats (Ranta 1975). The reduction in thyroxine secretion is probably due to a direct action on the pituitary and on the thyroid. Ferguson *et al.* (1965) reported a depression of 40% in wool growth following thyroidectomy in sheep. The thyroid depression observed here would tend to reduce wool growth but its contribution to the total wool growth depression observed in our experiments is yet to be determined.

We have concluded that large doses of dexamethasone caused shedding of the complete fleece in only some of the sheep in the experiments reported here without apparent deleterious effects on subsequent wool or body growth.

Acknowledgments

We are indebted to Dr M. Hichens for the antiserum to dexamethasone used in these studies. We also wish to thank Miss R. Jackson for her skilful analytical work and Mr S. G. Humphreys for his help with many aspects of the experiments.

References

- Arth, G. E., Fried, J., Johnston, D. B. R., Hoff, D. R., Sarett, L. H., Silber, R. H., Stoerk, H. C., and Winter, C. A. (1958). 16 α -Methylated steroids. II. 16 α -Methyl analogs of cortisone, a new group of anti-inflammatory steroids. 9 α -Halo derivatives. *J. Am. Chem. Soc.* **80**, 3161-3.
- Bassett, J. M., and Hinks, N. T. (1969). Microdetermination of corticosteroids in ovine peripheral plasma: effects of venipuncture, corticotrophin, insulin and glucose. *J. Endocrinol.* **44**, 387-403.
- Chapman, R. E., and Bassett, J. M. (1970). The effects of prolonged administration of cortisol on the skin of sheep on different planes of nutrition. *J. Endocrinol.* **48**, 649-63.
- Conney, A. H. (1967). Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* **19**, 317-66.
- Degroot, L. J., and Hoye, K. (1976). Dexamethasone suppression of serum T₃ and T₄. *J. Clin. Endocrinol. Metab.* **42**, 976-8.
- Eastman, C. J., Corcoran, J. M., Ekins, R. P., Williams, E. S., and Nabarro, J. D. N. (1975). The radioimmunoassay of triiodothyronine and its clinical application. *J. Clin. Pathol.* **28**, 225-30.
- Faglia, G., Ferrari, C., Beck-Peccoz, P., Spada, A., Travaglini, P., and Ambrosi, B. (1973). Reduced plasma thyrotrophin response to thyrotrophin releasing hormone after dexamethasone administration in normal subjects. *Horm. Metab. Res.* **5**, 289-92.
- Ferguson, K. A., Wallace, A. L. C., and Lindner, H. R. (1965). Hormonal regulation of wool growth. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 655-77. (Angus and Robertson: Sydney.)
- Hare, L. E., Yeh, K. C., Ditzler, B. S., McMahon, F. G., and Duggan, D. E. (1975). Bioavailability of dexamethasone. II. Dexamethasone phosphate. *Clin. Pharmacol. Ther.* **18**, 330-7.
- Haque, N., Thrasher, K., Werk, E. E., Knowles, H. C., and Sholiton, L. J. (1972). Studies on dexamethasone metabolism in man: effect of diphenylhydantoin. *J. Clin. Endocrinol. Met.* **34**, 44-50.
- Hemsley, J. A., Reis, P. J., and Downes, A. M. (1973). Influence of various formaldehyde treatments on the nutritional value of casein for wool growth. *Aust. J. Biol. Sci.* **26**, 961-72.
- Hichens, M., and Hogans, A. F. (1974). Radioimmunoassay for dexamethasone in plasma. *Clin. Chem. (Winston-Salem, N.C.)* **20**, 266-71.
- Lindner, H. R., and Ferguson, K. A. (1956). Influence of the adrenal cortex on wool growth and its relation to 'break' and tenderness of the fleece. *Nature (London)* **177**, 188-9.
- Panaretto, B. A., Chapman, R. E., Downes, A. M., Reis, P. J., and Wallace, A. L. C. (1975). Some effects of three glucocorticoid analogues on wool growth and their efficacy as defleecing agents in sheep. *Aust. J. Exp. Agric. Anim. Husb.* **15**, 193-202.
- Parke, D. V. (1976). The impact of drug metabolism on medicinal research. Part I. Survey of metabolic processes. *Chem. Ind. (London)* **1976**, 380-8.
- Ranta, T. (1975). Effect of dexamethasone on the secretion of thyrotrophin in the rat: dose and time relationships. *Endocrinology* **96**, 1566-70.
- Roberts, E. M., and McMahon, P. R. (1972). A study of the use of cyclophosphamide in Merino ewes as a means of fleece removal. *Proc. Aust. Soc. Anim. Prod.* **9**, 430-5.
- Ryder, M. L. (1971). Cycles of wool follicle activity in some Shetland sheep. *Anim. Prod.* **13**, 511-20.
- Sarett, L. H. (1959). Some aspects of the evolution of anti-inflammatory steroids. *Ann. N.Y. Acad. Sci.* **82**, 802-8.
- Singh, A., and Hardy, M. H. (1975). Effects of steroid hormones on developing mouse skin *in vitro*. *J. Endocrinol.* **66**, 195-205.
- Thwaites, C. J. (1972). The effects of short-term undernutrition and adrenocortical stimulation on wool growth. *Anim. Prod.* **15**, 39-46.
- Wilber, J. F., and Utiger, R. D. (1969). The effect of glucocorticoids on thyrotrophin secretion. *J. Clin. Invest.* **48**, 2096-103.

